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Structural Characterization of Mutant MoeB Proteins

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Introduction: The activation of ubiquitin and other protein modifiers is catalyzed by members of the E1 enzyme family, which use ATP for the covalent self-attachment of the modifiers to a conserved cysteine. The *Escherichia coli* proteins MoeB and MoaD are involved in molybdenum cofactor biosynthesis, an evolutionarily conserved pathway. The MoeB- and E1-catalyzed reactions are mechanistically similar, and despite a lack of sequence similarity, MoaD and ubiquitin display the same fold including a conserved carboxy-terminal Gly-Gly motif. Similar to the E1 enzymes, MoeB activates the C-terminus of MoaD to form an acyl-adenylate reaction intermediate.

Methods and Materials: All mutant MoeB/MoaD protein complexes crystallized in the tetragonal space group ($P4_12_12$) and were solved using difference Fourier methods.

Results: Based on our previous structural characterization of the MoeB/MoaD protein complex [1], we determined the structures of six different MoeB/MoaD mutant complexes. Two of these six mutants showed a significant decrease in activity (MoeB-R73K, MoeB-D130E) while a third mutant exhibited no detectable activity (MoeB-R14A/R73A). Crystals of each mutant complex were grown, harvested, and soaked with 10 mM ATP for 30 minutes prior to freezing and data collection to determine the ability of the mutant proteins to bind ATP. In all cases, the engineered mutations did not affect ATP binding to the active site cleft as evidenced by an obvious difference density corresponding to an ATP molecule. In a similar experiment, crystals of each mutant complex were soaked with 10 mM ATP and 10 mM $MgSO_4$ for 30 minutes prior to data collection. In the native crystals, the addition of Mg^{2+} is sufficient to produce the MoaD acyl-adenylate intermediate, however, the impaired activity mutants (MoeB-R73K, MoeB-D130E, MoeB-R14A/R73A) are incapable of forming the acyl-adenylate after a 30-minute incubation period.

Conclusions: These experiments have determined that the decreased activity seen for all impaired MoeB mutants is due to the inability of the mutant enzyme to form a MoaD acyl-adenylate intermediate, as all mutants are capable of binding an ATP substrate in a manner identical to the native MoeB protein.

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References:

[1] Lake, M. W., Wuebbens, M. M., Rajagopalan, K. V., Schindelin, H. (2001). Mechanism of Ubiquitin Activation Revealed by the Structure of a Bacterial MoeB-MoaD Complex. *Nature* **414,** 325-329.